

SUSCEPTIBILITY OF GYPSY MOTH LARVAE TO SEVERAL SPECIES OF ENTOMOGENOUS FUNGI

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Abstract.—Several species of fungal entomopathogens were tested for infectivity on larvae of the gypsy moth, *Lymantria dispar* (L.). Spore suspensions in varying dosages were administered to the larvae via surface treatment of sterilized oak leaves. High levels of mortality were obtained for most of the test species of fungi. *Conidiobolus thromboides*, *Fusarium* sp., *Hirsutella thompsonii*, one strain of *Paecilomyces farinosus* and *Verticillium* sp. were non-infective to gypsy moth larvae under the conditions of the experiment.

Introduction

The infectivity of entomogenous fungi on gypsy moth larvae has been demonstrated on the basis of qualitative and histopathological studies (Hartmann and Wasti 1974, 1976; Wasti and Hartmann 1975, 1978; Wasti et al. 1980). Toxicity tests on non-target invertebrate and vertebrate hosts have established the environmental safety of these candidate biological control agents (Donovan-Peluso et al. 1980; Hartmann and Wasti 1976, 1980; Hartmann et al. 1979; Wasti et al. 1980). Quantitative studies on the effect of specific dosage levels on mortality of gypsy moth larvae were the primary objectives of the present study.

Methods

Larvae of the gypsy moth, *Lymantria dispar*, were reared from eggs supplied by the Forest Insect Disease Laboratory, Hamden, Connecticut. Entomogenous fungal species tested for infectivity against these larvae were:

1. *Beauveria bassiana* (Bals.) Vuill. Russian strain
2. *Beauveria bassiana* (Bals.) Vuill. (*B. tenella*) ATCC 26156
3. *Cordyceps militaris* (Fries) Link ATCC 26848
4. *Conidiobolus thromboides* Drechsler (*Entomophthora virulenta*) (Hall & Dunn) Latge et al. (1980) ATCC 14270
5. *Fusarium* sp. (local strain)

6. *Hirsutella thompsonii* (Fisher) ATCC 24874
7. *Metarhizium anisopliae* (Metsch.) Sorokin ATCC 22099
8. *Paecilomyces farinosus* (Dicks. ex Fr.) Vuill. ATCC 24319
9. *Paecilomyces farinosus* (Dicks. ex Fr.) Vuill. USDA, Hamden, Ct. strain
10. *Paecilomyces fumoso-roseus* (Wize) Brown & Smith ATCC 16312
11. *Nomuraea rileyi* (Farlow) Samson, Missouri strain
12. *Verticillium* sp. (local strain)

Stock cultures of the various species of fungi were subcultured on Potato-dextrose agar and yeast extract (PDAYE) under procedures outlined by Hartmann and Wasti (1974). Culture isolates of the test species were then passed in serial succession through larvae of the wax moth, *Galleria mellonella* L. in order to increase virulence by passage through an easily inoculated host.

Infectivity studies were considered initially using the surface treatment technique recommended by Garcia and Ignoffo (1978) for infecting larvae with entomogenous fungi. Spore suspensions representing calibrated dosages of the fungal species were administered in sterile Petri dishes (dia. 60 mm) by spreading them over the surface of artificial gypsy moth diet under conditions recommended by O'Dell and Rollinson (1966). Results with this technique were unsatisfactory and the method proved unsuitable. There was difficulty in distributing low spore concentrations uniformly over the surface and infection rates with early instars were extremely low. Elimination of the antifungal agents in the diet increased the likelihood of infection by non-target saprotrophic fungi.

As an alternative, the leaflet technique described by Garcia and Ignoffo (1978) was used for the infectivity studies and was found to provide satisfactory results. Fresh oak leaves were surface-sterilized by immersion for several minutes in a one per cent solution of sodium hypochlorite and thoroughly rinsed with sterile distilled water. Appropriate dilutions of fungal spore suspensions were applied to the surface and each leaf was placed in a sterile Petri dish.

Fifty second instar larvae were surface-sterilized by immersion in a solution of Zephirine chloride (1:750) followed by a sterile water rinse, placed in each Petri dish, and allowed to feed on the leaves for 48 hours. Subsequently, the larvae were transferred to individual plastic chambers and observed at 24 hr intervals. The following spore dosages of each test organism were administered: 50, 100, 500, 1,000, 5,000 spores/mm². Untreated leaves were set up as controls and fed to a group of 100 2nd instar larvae used as controls. Each spore dosage was administered to 50 larvae.

Infected larvae were placed on sterile Petri dishes containing Potato Dextrose agar plus 0.5% yeast extract (PDAYE). One half gram Streptomycin

Table 1. Percentage mortality of gypsy moth larvae treated with surface applied suspensions of entomogenous fungi.

Dosage spores/mm ²	50		100		500		1,000		5,000	
	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr	72 hr	120 hr
<i>B. bassiana</i>	56	92	88	88	92	92	90	90	96	96
<i>B. tenella</i>	80	96	90	98	90	90	92	92	98	98
<i>C. militaris</i>	66	90	88	92	92	98	90	98	100	100
<i>M. anisopliae</i>	72	94	94	100	94	100	98	100	98	100
<i>N. rileyi</i>	80	96	90	100	98	100	100	100	92	100
<i>P. farinosus</i>	72	96	88	98	88	98	88	100	100	100
<i>P. fumoso-roseus</i>	84	100	88	100	92	100	96	100	88	100

and 0.5 g Penicillin G were added after the PDAYE was autoclaved for 15 min at 15 pounds pressure. The target organisms were recovered in all cases.

Results

The mortality rates of gypsy moth larvae to various dosages of entomogenous fungal spore suspensions have been summarized under Table 1. Control larvae showed 3% mortality after 72 hours and 5% mortality at the end of 120 hours. Of the 12 test species, 5 were considered non-infective under the conditions of the experiment. These were, *Conidiobolus thromboides*, *Fusarium* sp., *Hirsutella thompsonii*, *Paecilomyces farinosus* (Hamden, Ct. strain) and *Verticillium* sp.

Paecilomyces fumoso-roseus, *Metarhizium anisopliae* and *Nomuraea rileyi* were considered highly infective as 100% mortality was recorded within 120 hours of treatment with dosage levels as low as 100 spores per square millimeter of leaf surface. There was a linear dosage-mortality response and five of the seven infective species produced 100% mortality at the high dosages of 1,000 and 5,000 spores/mm². The target fungus was recovered from dead larvae and survivors did not show any adverse effects as a result of the treatment.

These tests simulate natural conditions as leaf surfaces were used for administration of the dosages. Entomogenous fungi exhibit varying levels of mortality against gypsy moth larvae and 5 of the 12 test species were non-infective.

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